

Appl. No. : 09/254,563  
Filed : March 5, 1999

Q1  
concd  
vitrification of cell or tissue specimens by dehydrating in solutions comprising a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant followed by cooling to refrigeration or higher storage temperatures.--

Q2  
Please insert the following sentence as the first sentence on page 1: -[This is a 35 U.S.C. §371 application of PCT/US97/15611, filed on September 5, 1997, which claims priority to U.S. Provisional application No. 60/025,570 filed on September 6, 1996.]

In the Claims

Please amend Claims 1, 4-7, 9, 10 and 12-16 as follows:

Q3  
sub B2  
1. (Amended) A method for preserving a cell or tissue specimen comprising [the steps of contacting]:

Q3  
N/M  
equilibrating and thereby dehydrating the specimen [with] in a vitrification solution comprising a permeating <sup>①</sup> cryoprotectant, a non-permeating <sup>①</sup> co-solute and a non-permeating <sup>③</sup> polymeric cryoprotectant, wherein the non-permeating co-solute effectively decreases the chemical potential of the [characterized by its ability to limit the amount of a] permeating cryoprotectant thereby limiting the amount of the permeating cryoprotectant which [to permeate] permeates into the specimen; and

Q3  
B  
vitrifying the dehydrated specimen, without freezing, by cooling to a refrigeration or higher storage temperature.

Q4  
4. (Amended) The method [for preserving a cell or tissue specimen as claimed in] of claim [2] 1, wherein the permeating cryoprotectant is selected from the group consisting of ① dimethylsulfoxide, ethylene glycol, propylene glycol and glycerol.

Q4  
5. (Amended) The method [for preserving a cell or tissue specimen as claimed in] of ③ claim [2] 1, wherein the non-permeating polymeric cryoprotectant is selected from the group consisting of dextrans, starches, polyethylene glycol, polyvinylpyrrolidone, [Ficol] FICOLL and peptides.

Q4  
② 6. (Amended) The method [for preserving a cell or tissue specimen as claimed in] of claim 1, wherein the non-permeating co-solute is selected from the group consisting of an amino acid, [and] an amino acid derivative [derivatives thereof], a betaine, a carbohydrate and a sugar alcohol, wherein the carbohydrate is selected from the group consisting of an aldose

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monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic, uronic and aldaric acids, disaccharides and polysaccharides].

Sub B3  
7. (Amended) The method [for preserving a cell or tissue specimen as claimed in] of claim 1, wherein the total concentration of non-permeating co-solute [in the co-solute solution] is between about 0.1 and 0.7 mol/L [and is equal to a maximum possible concentration that does not substantially damage cells].

Sub B4  
9. (Amended) The method [for preserving a cell or tissue specimen as claimed in] of claim [2] 1, wherein [the method] equilibrating and thereby dehydrating the specimen is performed in two or more stages of contacting the [sample] specimen with increasingly higher concentrations of the permeating cryoprotectant and the co-solute.

10. (Amended) The method [for preserving a cell or tissue specimen as claimed in] of claim [2] 1, wherein [the method] equilibrating and thereby dehydrating the specimen is performed by simultaneously increasing concentrations of both permeating cryoprotectant and the co-solute from an initial concentration to a final concentration according to a desired profile.

Sub B5  
12. (Amended) The method [for preserving a cell or tissue specimen as claimed in] of claim [11] 1, further comprising [the step of] rehydrating the specimen by contacting the [preserved] vitrified specimen with a rehydration solution comprising a non-permeating rehydration co-solute [characterized by its ability to limit the amount of a] which effectively decreases the chemical potential of the permeating cryoprotectant [to permeate into the specimen, such that cryoprotectant within the specimen is removed from the cells of the specimen].

13. (Amended) The method [for preserving a cell or tissue specimen as claimed in] of claim 12, wherein the permeating rehydration cryoprotectant is selected from the group consisting of dimethylsulfoxide, ethylene glycol, propylene glycol and glycerol.

14. (Amended) The method [for preserving a cell or tissue specimen as claimed in] of claim [12] 13, wherein [the rehydration step] rehydrating the specimen is performed by simultaneously decreasing concentrations of both the permeating rehydration cryoprotectant and the non-permeating rehydration co-solute from an initial concentration to a final concentration according to a desired profile.

15. (Amended) The method [for preserving a cell or tissue specimen as claimed in] of claim 12, wherein the non-permeating rehydration co-solute is selected from the group consisting of an amino acid, [and] an amino acid derivative [derivatives thereof], a betaine, a carbohydrate